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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

We are comparing vascular endothelial cells isolated from 1) the ends of bone where metastasized breast cancer cells frequently lodge and proliferate (cells called BVECs) and from 2) the nearby central marrow cavity (cells called MVECs). Project-1: to acquire a broader view of differences between BVECs and MVECs, one goal is to compare messenger RNA using microarray analysis. We have purified the cell populations by flow cytometry; this resulted in impaired RNA quality. We have set up a magnetic bead method to more gently isolate and purify the cells using an antibody to PECAM, a protein specifically on vascular endothelial cells. Project-2: we have found differences in expression and display of surface adhesion molecules. Using immunodetection, BVECs express more total p-selectin, e-selectin, ICAM-1, and VCAM-1 than MVECs; only a few (5-15%) of both cell types display these adhesion molecules externally. BVECs display more surface e-selectin when exposed to conditioned medium from MDA-MB-435 breast cancer cells. BVECs are fully stimulated by osteoblast conditioned medium, but MVECs respond by displaying substantially more p-/e-selectins. The data, while preliminary, suggest that BVECs in the ends of long bones possess adhesion properties that favor the entrapment of breast cancer cells.

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Introduction

The goal of this research is to identify characteristics of the cellular environment in the ends of long bones that fosters the entrapment and proliferation of metastasized breast cancer cells. Studies published by others indicate that the vascular cells from skeletal biopsies present more surface adhesive proteins than do other vascular cells (Lehr & Pienta, 1998). We have developed methods to isolate vascular cells from the ends of long bone and are comparing expression of surface adhesive proteins between the bone vascular endothelial cells (BVECs) and analogous cells in the nearby marrow compartment (MVECs) using immunodetection and microarray analysis.

Body

Task 1. Compare endothelial cells of the microvasculature from the ends of long bone (BVECs) and analogous cells from the central marrow cavity (MVECs). We have isolated both BVECs and MVECs from 30 mice in two pools, allowed the cells to recover and expand for 10 days in culture. The cells were then allowed to take up acetylated-LDL tagged with fluorescent alexa 488, released from the culture flask with 0.025% trypsin and processed by flow cytometry. Approximately 1.2×10^6 BVECs and 1.7×10^6 MVECs were obtained. The cells were collected in Medium 199 and recultured for 5 days to allow the cells to repair possible damage incurred during isolation. These cells grew poorly in culture, however control cells treated identically, but not subjected to flow cytometry proliferated robustly in culture. This points to flow cytometry as being a problem for isolating robust, viable endothelial cells. The RNA obtained by extraction with Qiagen RNeasy was of poor quality and low yield, apparently due to damage caused by flow cytometry. We have now implemented a magnetic bead method to more gently isolate the cells. This involves tagging the cells with a specific biotinylated antibody (anti-PECAM) since PECAM is specifically found on vascular endothelial cells. Next, the cells are exposed to iron-containing micro-beads that are coated with avidin. The cell preparation is placed in a column of beads in a magnetic field. The columns are rinsed repeatedly with culture medium. Non-specific, untagged cells flow out of the column, leaving a highly pure fraction of vascular cells held in place by the magnetic field. The

selected cells are then released when the magnetic field is abolished. We have not progressed yet to the RNA extraction phase for this new set of cells.

Task 2. Determine the difference in the attraction of breast cancer cells to the two types of vascular cells. We have found differences in expression of surface adhesive proteins in the bone vascular endothelial cells (BVECs) as compared to marrow vascular endothelial cells (MVECs). By applying antibodies to isolated BVECs and MVECs we have found that BVECs express more total (internal and surface) p-selectin, e-selectin, ICAM-1 and VCAM-1 than do MVECs. A small fraction of both types of cells, ranging from 5-15%, display these proteins on their surfaces. When cultured with osteoblast conditioned medium the BVECs change minimally, suggesting that expression of surface proteins is already maximized. MVECs do respond to osteoblast conditioned medium with more cells displaying p-selection and e-selection. This suggests that MVECs can become more like BVECs under the right conditions. A profound increase on surface e-selection occurred for BVECs under the influence of conditioned medium from the MDA-MD-435 breast cancer cells. Initial evidence suggests that BVECs are more likely to be attractive (i.e. serve as an adherent surface) to breast cancer cells than MVECs.

Key Research Accomplishments

- Scaled up vascular cell isolation method using flow cytometry. RNA obtained was of poor quality and low yield.
- Set up a magnetic bead method to more gently isolate cells on a scale to efficiently isolate and purify BVECs and MVECs for RNA isolation.
- Compared surface adhesive proteins on BVECs and MVECs. Breast cancer cells stimulate BVECs deployment of e-selection.

Reportable Outcomes

All results need to be repeated and subjected to statistical analysis.

Conclusions

We are in the process of modifying the approach of obtaining RNA from bone vascular cells (BVECs) and central marrow cavity vascular cells (MVECs). Conclusions are not yet possible.

We have made headway in identifying differences in certain surface adhesive proteins between BVECs and MVECs. BVECs appear to maximally display surface p-selection, e-selection, ICAM-1 and VCAM-1 making them a better attractive surface for breast cancer cells than do MVECs.

BVECs produce more surface e-selectin in response to breast cancer cell conditioned medium, a response that increases their attractiveness to breast cancer cells.

References

Lehr J.E. and Pienta K.J. 1998. Preferential adhesiion of prostate cancer cells to a human bone marrow endothelial cell line. J. Natl. Cancer Inst. 90: 118-123.